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Synthesis, Spectral, Anticancer Studies on Cu (II) Complex of 2,4,5-Triphenyl-1*H*-Imidazole

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Abstract

Schiff bases of 2,4,5-triphenyl-1*H*-imidazole ligand and Cu (II) complex has been synthesized and characterized by ^{1}H and ^{13}C NMR, elemental analyses, molar conductance, thermal analysis (TGA), magnetic moment measurement, IR and UV-Vis. On the basis spectral studied and analytical data, it is evident that the Schiff base acts as monodentat ligand coordinating via nitrogen atom. The result showed that Cu (II) complex has octahedral geometry. The studied complex was tested for anticancer activity. The anticancer activity of Cu (II) complex is evaluated against liver Carninoma (HEPG2) cell lines. These compounds exhibit a moderate and weak activity against HCT 116 cell line with IC_{50} 19.8 μM.

***Keywords*:** Schiff bases, anticancer, imidazole, octahedral geometry

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1. INTRODUCTION

Imidazole derivatives have been shown to exhibit interesting biological activities, including anti-allergy, anti-tumor, anti-inflammatory, anti-bacterial, and analgesic (Shelke *et al.,* 2009; Wang *et al.,* 2001; Mohammadi *et al.,* 2012; Joshi *et al.,* 2010; Ucucu *et al.,* 2001). Hence, imidazole plays an important role in biochemistry, medical science, non linear optical materials and so on. Researches on complex compounds as anticancer have been developed until now many studies of new anticancer drugs made of metal that have been reported. Metal attached directly to an organic compound is called organomettalic compounds. Organomettalic complex can be classified based on cytotoxic activity against cancer cells by looking at the chemical-physical processes that enable the interactions toward biological targets (Sadler, 1991).

In some studies, the imidazole derivatives are widely used as ligands in the formation of complex compounds. Imidazole derivatives provide good antimicrobial activity. One of the examples is the formation of complex with the 2-(4-thiazolyl)-1*H*-benzimidazole and benzimidazole ligand. 2-(4-thiazolyl)-1*H*-benzimidazole compound has antimicrobial and antifungal activity higher than benzimidazole (Rahmapreet *et al.,* 2011). It happened because 2-(4-thiazolyl)-1*H*-benzimidazole has more aromatic substituents. Imidazole derivatives with the substitution of fluorine, propylene, and aromatic tetrahydroquinoline can improve stability, bioactivity and biological activity. 2,4,5-triphenyl-1*H*-imidazole has a structure that allows it to be a bridging ligand so that the possibility to form complex polymers are also quite high (Walia, 2011). The polymer complexs have very good stability. 2,4,5-triphenyl-1*H*-imidazoles can be used as a corrosion inhibitor as reported (Wahyuningrum, 2008). However, only few studies discuss its biological activity and toxicity.

Therefore, the present work aims to synthesize Schiff base ligand 2,4,5-Triphenyl-1*H*-Imidazole and its complex with Cu(II) ion.

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The complex has been characterized by several tools of analyses such as ^{1}H and ^{13}H NMR, elemental analyses, molar conductance, thermal analysis (TGA), magnetic moment measurement, IR and UV-Vis spectra. Also, anticancer activity of the synthesized compound has been studied.

2. MATERIAL AND METHODE

Materials

All compounds used in the present study were of pure grade available from Merck. The solvents used for the spectral studies were spectroscopic grade from Aldrich.

Instrumentations

The elemental microanalyses of the solid compound was performed at the micro analytical center, ^{1}H-NMR and ^{13}C-NMR Jeol 500, University Kebangsaan Malaysia using Perkin Elmer 2400 CHN Elemental analyzer, Metal content was estimated using Atomic Absorption Spectroscpy (AAS) ZEEnit 700, Molar conductivities in DMF (10^{-3} M) at room temperature (27 °C) were measured using Mettler Toledo, The infrared spectra were recorded using 8400S Shimadzu FTIR spectrophotometer within the range 4000-400 cm^{-1} as KBr discs. The electronic absorption were recorded using a genesys 10S UV-Vis spectrophotometer. The room temperature magnetic susceptibility of the solid samples was measured using magnetic susceptibility balance (MSB) Sherwood Scientific LTD 1 employing the Gouys method. The thermogravimetric analysis (TGA) of the solid samples was performed within the temperature range 25-500 °C using the STAR SW 10.00 thermogravimetric analyzer with heating rate (10 °C/min) under nitrogen atmosfir.

Synthesis of 2,4,5-triphenyl-1*H*-imidazoles Ligand

The Schiff base ligand (Fig. 1) was prepared by the reflux of benzyl (0.82 g, 3.8 mmol), (1.52 g, 19.7 mmol) ammonium acetate and 0.3 mL benzaldehyde in 30 mL acetate acid. A mixture of them was refluxed for 2.5 h. The reaction mixture was then poured onto ice water. The product which separated immediately was filtered off, washed several times with cold water and finally dried in a vacuum desiccators over anhydrous calcium chloride.

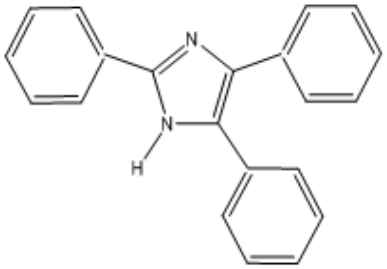

Figure 1. The structure of Schiff base 2,4,5-triphenyl-1*H*-imidazoles ligand

Synthesis of Complex Cu(II) with 2,4,5-triphenyl-1*H*-imidazoles Ligand

To a solution of ligand (0.29 g, 1 mmol) in 20 ml methanol, a methanol (20 ml) of the hydrated Cu (II) nitrate (0.12 gram, 0,5 mmol $Cu(NO_3)_2 \cdot 3H_2O$) was added slowly with constant stirring. The reaction mixture was heated to reflux for 24 h. The resulting mixture was filtered off and then left to evaporate in a beaker in air at ambient temperature. Green crystal were formed within several days.

3. RESULTS AND DISCUSSION

Elemental Analysis and Atomic Absorption Spectrophotometer Analysis

Levels of metal ions Cu (II) complex synthesis product is determined by atomic absorption spectroscopy instruments. Snippets can be measured by the instrument SSA tangible solution. The complex is not soluble in distilled water. The compound was destructed in HNO_3 solution, then added with aquademin.

Table 1. Result of elemental analysis complex Cu(II) with 2,4,5-triphenyl-1*H*-imidazoles

Complex	% C		% H		% N		% Cu	
	Found	Calculated	Found	Calculated	Found	Calculated	Found	Calculated
$[CuL_2(H_2O)_2](NO_3)_2$	61.27	61.84	4.83	4.42	10.67	10.31	7.59	7.73

L = 2,4,5-triphenyl-1*H*-imidazole

The result of the elemental analysis on the precipitated complex are reported in Table 2. Calculated and found element percent are in good agreement. It can be noted that the complex do not have the external water molecules even if the experimental conditions previously reported were strictly followed. It can be due to the drying process that removes all the unstructured water molecules.

Magnetic Moment Measurement

The magnetic moment data of the solid complexes at room temperature showed that all complex are paramagnetic. The magnetic moment value of Cu (II) complex (1.96 BM) is higher than the theoretical spin only value of Cu (II) complex refers to spin orbital coupling. These values indicate octahedral structure for complex Cu (II).

FT-IR

The most important IR bands of 2,4,5-triphenyl-1*H*-imidazoles ligand with their assignments are presented in fig 2. The ligand spectrum showed 3080, 1602, 1949 and 765 cm^{-1} attributed, respectively to (CH, C=C, overtone aromatic, out of plane aromatic). Complex compounds [Cu(II)-2,4,5-triphenyl-1*H*-imidazole $(H_2O)_2](NO_3)_2$ measured at wave number 3000-500 cm^{-1}. This spectrum is used to determine the presence of functional groups in a complex compound that can assist in predicting the structure of complex compounds. In FTIR spectra, peaks widened at wave number 1600-1700 cm^{-1} which indicates the presence of C-N bonded to the metal Cu with coordination ligand 2,4,5-triphenyl-1*H*-imidazole. Peak in the area of 3039.60 cm^{-1} is a C-H bond stretching aromatic peak. In addition 1600-1700 cm^{-1} is a vibration of C-N ligand 2,4,5-triphenyl-1*H*-imidazole with coordination Cu logam. Cu-N bond is shown in wave numbers 422.38 cm^{-1}. In the FTIR spectrum, NO_3 bond of synthesized complex [Cu(II)-2,4,5-triphenyl-1*H* imidazole]$(NO_3)_2$ appears at wave number 1400 cm^{-1}. While the Cu-O bond is observed at 534.25 cm^{-1} region.

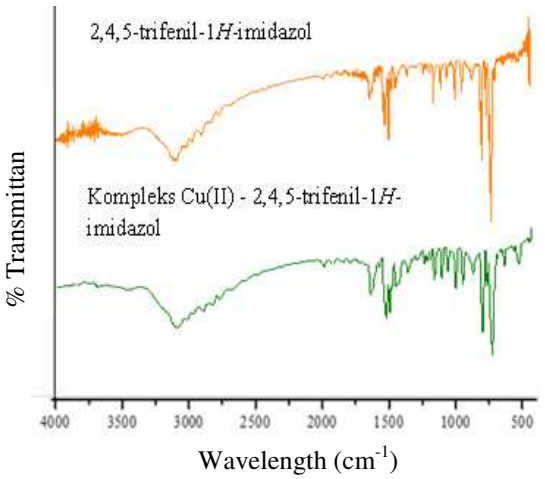

Fig 2. IR Spectra of the 2,4,5-triphenyl-1*H*-imidazoles ligand and complex Cu(II) with 2,4,5-triphenyl-1*H*-imidazoles ligand

NMR (^{1}H and ^{13}C) Spectra

Its ^{1}H and ^{13}C NMR spectrum of 2,4,5-triphenyl-1*H*-imidazoles ligand was recorded in d^6-DMSO reported in table 1 and showed in figure 3 for ^{1}H NMR figure 4 for ^{13}C NMR.

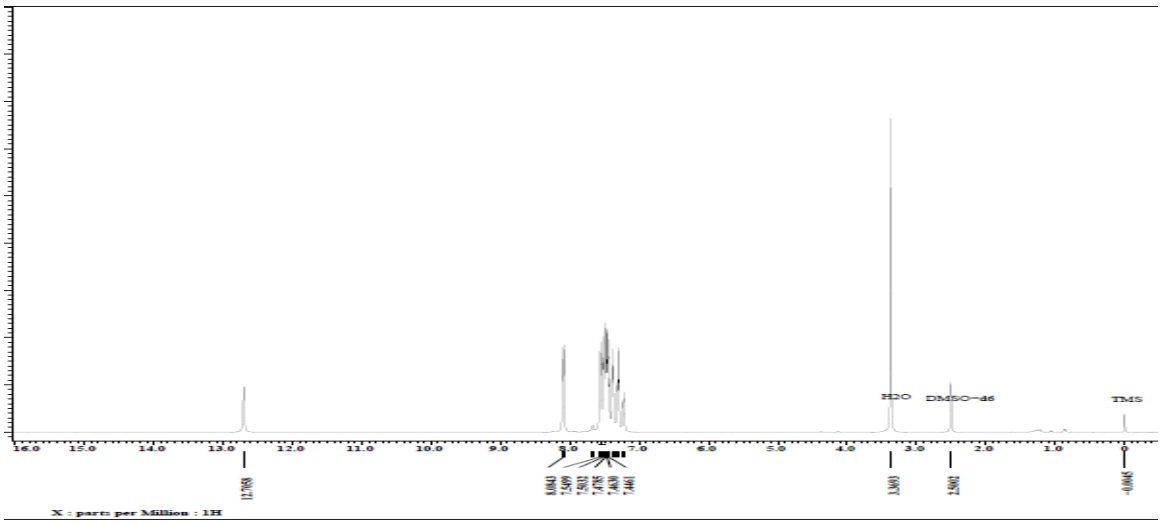

Figure 3. ^{1}H NMR of the 2,4,5-triphenyl-1*H*-imidazoles ligand

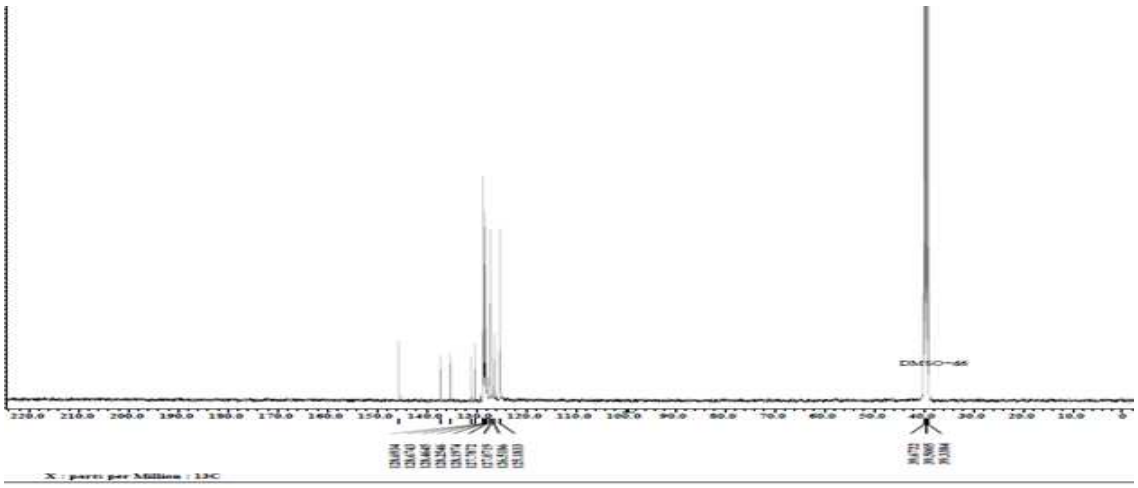

Figure 4. ^{13}C NMR of the 2,4,5-triphenyl-1*H*-imidazoles ligand

Table 2. ^{1}H NMR and ^{13}C NMR data of the 2,4,5-triphenyl-1*H*-imidazoles ligand

^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
12.70 (s, 1H, NH) 8.09 – 8.08 (d, 2H) 7.56 – 7.55 (d) 7.52 – 7.43 (m) 7.31 – 7.36 (t) 7.32-7.29 (t) 7.24-7.21 (t)	145.5; 137.1; 135.2; 131.1; 130.3; 128.7; 128.6; 128.5; 128.3; 128.2; 127.8; 127.1; 126.5; 125.2

Molar Conductance

Table 3. Molar conductance of Cu (II) complex

Compound	Λ_m (S.cm^2.mol^{-1})	Electrolyte
Methanol	4.75	-
KCl in methanol	98.57	1 : 1
$MgCl_2$ in methanol	157.45	2 : 1
$FeCl_3$ in methanol	258.85	3 : 1
Complex Cu(II) in methanol	161.05	2 : 1`

Thermogravimetry Analysis

Thermogravimetric analysis was used to estimate species that decompose or left. It can be determined by comparing the weight of the sample at a temperature of decomposition to the initial weight. The results of analysis by Thermal Gravimetric Analyser (TGA) of complex compounds Cu (II) is shown in Figure 5. The analysis was performed with a heating rate of 10 ºC/h at 20-600 ºC and the initial sample weight of 23.7 mg.

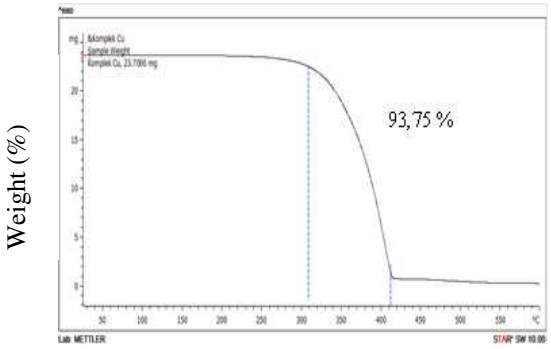

Figure 4. Thermogravimetry analysis of complex Cu (II) with 2,4,5-triphenyl-1*H*-imidazole

Based on the results of this analysis, it can be seen that the decomposition does not happen at temperature 120-150 ºC which indicates that the complex does not contain crystal water. Decomposition peak appeared at between 291.55-417.47 ºC. At this temperature decomposition was as much as 93.75%. At this temperature it was predicted that mass loss was metal, the two ligand 2,4,5-triphenyl-1*H*-imidazole, two water ligands, and HNO_3 ligand. The predictions of complex compounds of formula [Cu(II)-2,4,5-triphenyl-1*H*-imidazole $(H_2O)_2](NO_3)_2$.

Toxicity Test

Toxicity tests were performed using the BSLT (Brine Shrimp Lethality Test)

method. Experimental animals used in this method is the larval of Artemiasalina shrimp. The LC_{50} values will be obtained from this test. This value is used to determine the toxicity of a compound. Toxicity tests were performed using the BSLT (Brine Shrimp Lethality Test) method (Meyer, 1982). In this test, variations in the concentration used is 25 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm. The measurement was performed with three repetitions (triplo) for each concentration .Data on the number of deaths in each of various concentrations shown in Table 4. Then log concentration against % mortality graph is shown in Figure 4 .

Table 4 . LC_{50} value with BSLT method

Concentration ppm	Log	Dead	Life	% Mortality
25	1.40	0	31	0.00
50	1.70	2	30	2.02
100	2.00	6	25	10.67
250	2.40	8	24	27.59
500	2.70	12	18	60.87
1000	3.00	31	0	100.00

Through calculations using polynomial regression equation in Figure 5, it is known that the LC_{50}values is 389.04 mg/L (complete calculation in appendix). LC_{50} value of the complex Cu (II)-2,4,5-triphenyl-1*H*-imidazole proven research (Walia *et al.,* 2011) that the more aromatic compounds in the complex can enhance the stability and biological activity. LC_{50} value obtained is greater than the value of the pure compound toxicity limit which is 200 ppm.

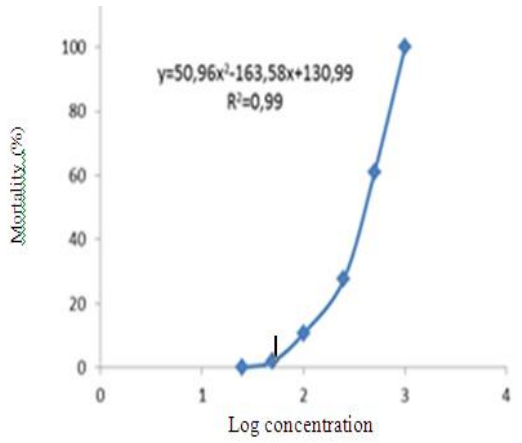

Figure 5. BSLT (Brine Shrimp Lethality Test)

Anticancer Activity

Cu (II) complex with 2,4,5-triphenyl-1*H*-imidazole ligand has been sought for anticancer treatment. The values of IC_{50}, compared with the standard drug doxorubicin (Drox) the liver Carninoma (HEPG2) are collected in Table 4. Untreated cells were used as a control. The ligand and Cu(II) complex showed an inhibition of cell viability and gave the IC_{50} value 8.7 μM and 19.8 μM against HEPG2, respectively, compared with IC_{50} value of 4.73 μM for the standard cytotoxin drug doxorubicin.

4. CONCLUSION

A schiff base of 2,4,5-triphenyl-1*H*-imidazole ligand and Cu(II) complexes were synthesized and characterized by ^{1}H and ^{13}C NMR, elemental analyses, molar conductance, thermal analysis (TGA), magnetic moment measurement, IR and UV-Vis. On the basis spectral studie and analytical data, it is evident that the Schiff base acts as monodentat ligand coordinating via nitrogen atom.. The Cu(II) complex have octahedral geometry. The kinetic and thermodynamic parameters of the thermal decomposition stages have been evaluated using Coats. Redfern method. In vitro anticancer activity of these complexes showed IC_{50} 19.8 μM.

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